Comparison of Constrictor Responses of the Rabbit Ear Artery to Norepinephrine and to Sympathetic Nerve Stimulation

By John G. Waterson

ABSTRACT

The perfused isolated rabbit ear artery was used as a vascular model to compare the constrictor responses to exogenous and endogenous norepinephrine. The aim of the study was to estimate the concentrations of norepinephrine reaching the smooth muscle of the artery during sympathetic nerve stimulation. The artery was perfused so that exogenous norepinephrine was introduced exclusively from the adventitial surface; thus, the drug followed a path to the smooth muscle layer similar to that of the endogenous norepinephrine released from the perivascular ring of sympathetic nerves during electrical stimulation of the artery wall. Cocaine was used to remove the influence of neural uptake of norepinephrine on concentrations of the amine reaching the muscle receptor sites. The estimated concentrations of norepinephrine reaching the smooth muscle receptors for each pulse of electrical stimulation ranged from 24.8 ± 5.9 ng/ml (1.47 × 10⁻⁷ M) to 29.6 ± 7.7 ng/ml (1.75 × 10⁻⁷ M) over the range of pressure changes from 30 to 100 mm Hg. This finding suggests that the relative sensitivity to endogenous and exogenous norepinephrine is similar at different response levels in this range and that the effect of local factors in the artery on concentrations reaching the smooth muscle is of the same order of magnitude for both endogenous and exogenous norepinephrine.

KEY WORDS artery constriction smooth muscle transmitter concentrations perfused artery catecholamines cocaine

Informative studies on the release of norepinephrine from sympathetic nerves were carried out using the spleen (1, 2), and they attempted to estimate the amounts of norepinephrine released by electrical stimulation of the sympathetic nerves. Similar studies were made on blood vessels to estimate the amount of norepinephrine released per impulse of nerve stimulation from the vascular sympathetic nerves in cat skeletal muscle (3). In parallel studies, Folkow and Haggendal (4) estimated the concentrations of norepinephrine reaching the neuroeffector junction in cat vascular smooth muscle, and later Ljung (5) estimated local transmitter concentrations in the vascular smooth muscle of the rat portal vein during vasoconstrictor activity.

One method of indirectly estimating local transmitter concentrations attained in vascular smooth muscle is to compare the constrictor responses to endogenous nerve-released norepinephrine with those to exogenous norepinephrine. A major disadvantage of this method is that in many tissues, e.g., rat portal vein, the sympathetic nerves are contained within the smooth muscle mass, and endogenous norepinephrine released from the nerves follows a different path to the smooth muscle receptor sites than does exogenous norepinephrine, which enters the smooth muscle from an external source.

In the isolated rabbit ear artery preparation, the sympathetic nerves are confined to a region outside of the smooth muscle mass (6). When the artery is perfused with exogenous norepinephrine applied to the adventitial surface only, then exogenous and endogenous norepinephrine follow similar paths to the receptor sites in the smooth muscle layer of the artery.

In the present study, a comparison has been made of the constrictor effects of exogenous norepinephrine (bathing the artery during perfusion) and endogenous norepinephrine to estimate the concentrations of transmitter reaching the smooth muscle receptor sites during prolonged sympathetic nerve stimulation.
An important mechanism for eliminating norepinephrine from synaptic regions is uptake into sympathetic nerves by a membrane pump mechanism (7), and it is possible that such a mechanism could affect the concentrations of both exogenous and endogenous norepinephrine reaching the smooth muscle during the comparison of their vasoconstrictor activities. It was shown in an earlier study that the neural uptake process in the rabbit ear artery reduced the magnitude of constrictor responses to exogenous norepinephrine (8). Therefore, in this study, the influence of the neural uptake process on concentrations of exogenous norepinephrine in the vicinity of the sympathetic nerves was removed by the use of cocaine.

Methods
Semilop-eared rabbits bred at the Central Animal House, University of Adelaide, were used in the study. Central arteries were removed from the ears under urethane anesthesia as described by de la Lande and Rand (9) and perfused using the double-cannulation method described by de la Lande et al. (10). With this method drugs may be added separately to either the intraluminal or the extraluminal surface. Arteries were perfused using a roller pump delivering a constant volume (5–6 ml/min). Perfusion pressure was monitored with an Ether pressure transducer and a Rikadenki chart recorder. Constriction of the artery was shown as an increase in perfusion pressure.

The composition of the perfusion fluid (in g/liter was as follows: NaCl 6.9, KCl 0.35, CaCl₂ 0.28, MgCl₂ 0.1, NaHCO₃ 2.1, KH₂PO₄ 0.16, and glucose 1.0.

EXPERIMENTAL PROCEDURE
Arteries were perfused with Krebs-bicarbonate solution bubbled with 5% CO₂ in oxygen and maintained at 37°C. Perfusion continued for at least 30 minutes before the application of drugs or electrical stimulation.

The arteries were exposed to two sets of conditions causing constriction. (1) Various concentrations of norepinephrine were applied to the adventitial surfaces so as to cause graded increases in perfusion pressure. The drug remained in contact with the artery until a maximal response was reached. The drug was then washed out with Krebs solution. (2) Arteries were stimulated through periartrial electrodes as described by de la Lande and Rand (9). An Eilco type 6418 stimulator was used. Voltage was supramaximal, and pulse frequency varied from 1 to 20 pulses/sec, with a duration of 0.3 msec. The current was applied until a maximal constriction was reached.

Cocaine (1 μg/ml) was applied to the artery by adding it to both the intraluminal and the extraluminal bathing fluids. Cocaine remained in contact with the artery for at least 10 minutes before further constrictor responses were measured. Norepinephrine and electrical nerve stimulation were again applied to the artery. Constrictor responses consisting of pressure increases in the range of 10 to 150 mm Hg were obtained. The constrictor responses were again measured as the maximal contraction obtained in a prolonged response. Dose-response curves were prepared after measuring two or more responses to a particular stimulus with at least two stimulus levels.

![Graph](http://circres.ahajournals.org/)

**FIGURE 1**

Left: Typical responses to external norepinephrine (ng/ml). Right: Typical responses to prolonged electrical stimulation (pulses/sec) at 75 v, 0.3 msec.
ARTERIAL NEUROTRANSMITTER CONCENTRATIONS

**Results**

**RESPONSES TO NOREPINEPHRINE**

The constrictor responses to norepinephrine were either monophasic or biphasic and have been described in earlier reports (8, 11).

A typical constrictor response is shown in Figure 1. Increases in perfusion pressure were determined by measuring the maximal height attained during a prolonged response, usually reached within 2 minutes of application of the drug. Dose-response curves were constructed for each experiment, and a typical curve is shown in Figure 2. In some experiments dose-response curves were made prior to and during the presence of cocaine to estimate the potentiation of the response to extraluminal norepinephrine. The potentiation caused by cocaine was three- to tenfold (eight arteries).

From the dose-response curves the concentrations of norepinephrine which increased the perfusion pressure by 30, 60, and 100 mm Hg were estimated by linear interpolations. The means of the estimates at each of the three perfusion pressures are shown in Table 1. Where an estimate could not be made at any one of the three pressure levels, no figure was recorded; this procedure accounts for the absence of estimates at some points in Table 1. In no case was an extrapolation of a dose-response curve made.

**RESPONSES TO NERVE STIMULATION**

The responses to nerve stimulation were qualitatively similar to those described for norepinephrine. The application of cocaine resulted in the slight potentiation noted in the rabbit ear artery by previous workers (9, 12). A typical response to nerve stimulation is shown in Figure 1. Dose-response curves were drawn by plotting increases in perfusion pressure against frequency of stimulation, and a typical curve is shown in Figure 2.

Estimates were made by interpolation of the number of pulses/sec necessary to raise the perfusion pressure by 30, 60, and 100 mm Hg. The mean values in number of pulses/sec for attainment of these pressures are shown in Table 1. Table 1 also contains the ratio of norepinephrine concentration (in ng/ml) to nerve stimulation (in pulses/sec) for 30-, 60-, and 100-mm Hg increases in perfusion pressure.

**Discussion**

Previous studies have been conducted to determine quantities of transmitter substance released from the rabbit ear artery (13-15). In the present study the concentrations of norepinephrine causing constriction of the rabbit ear artery were compared with the frequencies of electrical nerve stimulation causing similar constrictions. The validity of comparing norepinephrine application with nerve stimulation is strengthened by considerable evidence.
TABLE 1

Effects of Nerve Stimulation and Exogenous Norepinephrine

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<th>Artery no.</th>
<th>PULSES/sec</th>
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<th>60 mm Hg</th>
<th>100 mm Hg</th>
<th>NE (ng/ml) 30 mm Hg</th>
<th>60 mm Hg</th>
<th>100 mm Hg</th>
<th>NE/ES ratio (ng/ml/mM)</th>
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<th>60 mm Hg</th>
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The numbers indicate the pulses/sec and the concentration of norepinephrine (NE) in ng/ml which raised the perfusion pressure by 30, 60, and 100 mm Hg. The ratio of norepinephrine added to frequency of stimulation (NE/ES) is also shown. Grouping of two consecutive artery numbers indicates that these arteries came from the right and left ears of the same rabbit.

The ratio of norepinephrine (ng/ml) to electrical stimulation (pulses/sec) in Table 1 shows estimates of the concentration of norepinephrine necessary to constrict the artery to the same extent as 1 pulse/sec of nerve stimulation at each of the three pressure levels chosen, namely, 30, 60, and 100 mm Hg. The means of the ratios at each pressure level are shown in Table 1, and using these values hypothetical figures for effective concentrations of norepinephrine reaching smooth muscle receptors were arrived at. The estimated concentrations for each pulse of electrical stimulation are: (1) 27.5 ± 9.08 (SE) ng/ml (1.63 × 10⁻⁷M) for a 30 mm Hg rise, (2) 24.8 ± 5.9 ng/ml (1.47 × 10⁻⁷M) for a 60 mm Hg rise, and (3) 29.6 ± 7.7 ng/ml (1.75 × 10⁻⁷M) for a 100 mm Hg rise. The estimates are speculative, since several assumptions were made in arriving at the figures.

One of the results (concentration of endogenous norepinephrine at the receptor sites) depends on the assumption that norepinephrine concentrations in the organ bath are those which finally reach the smooth muscle receptors after passing through the adventitia and the neural and smooth muscle layers. Factors which may affect this final concentration are: (1) enzyme activity and tissue binding in the central artery of the rabbit ear was provided by fluorometric assay (15) and by histochemical studies (6, 17).
adventitia, (2) neural uptake of norepinephrine, and (3) enzyme activity and smooth muscle binding of norepinephrine in the media.

Lowering of the norepinephrine concentrations by enzyme activity and tissue binding in the adventitia could be important in comparisons of endogenous and exogenous norepinephrine, since endogenous norepinephrine can reach the smooth muscle receptors without passing through the adventitia, but exogenous norepinephrine applied to the adventitial surface must traverse the adventitial tissue before reaching the smooth muscle mass. However, the responses were measured after prolonged contact with norepinephrine, and, if tissue binding occurred in the adventitia, saturation was probably reached by the time the maximal response height was achieved. If enzyme induction occurred in this region, concentrations in the adventitia would fall by virtue of enzyme activity, but again the prolonged contact of relatively high concentrations of norepinephrine would create conditions which are not conducive to major changes in concentrations of exogenous norepinephrine.

Neural uptake can be disregarded as an important factor affecting exogenous norepinephrine, since cocaine was present in concentrations known to prevent neural uptake.

Enzyme activity and smooth muscle binding may affect both exogenous and endogenous norepinephrine during diffusion of the amine into the smooth muscle, and it must be conceded that a lowered concentration of amine caused by loss in this region would tend to reduce the estimate of the concentration of norepinephrine equivalent to 1 pulse/sec of nerve stimulation. However, evidence has been presented (11) that only a relatively slight penetration of norepinephrine into the smooth muscle layer of the rabbit ear artery is necessary to produce large constrictor responses. Thus, enzyme degradation and smooth muscle binding of norepinephrine, although known to occur in the rabbit ear artery (18), probably were not major factors affecting the estimation of concentrations of endogenous and exogenous norepinephrine in the present study.

The ratios of norepinephrine concentration to the pulses/sec of nerve stimulation at each of the three chosen levels of pressure increases were remarkably similar. This finding suggests that the relative sensitivity to endogenous and exogenous norepinephrine undergoes little change over the range of pressure increases between 30 and 100 mm Hg and that, if local factors in the artery wall are affecting concentrations, the effect is of the same order of magnitude for both endogenous and exogenous norepinephrine.

In previous studies measurements were made of the total amounts of norepinephrine released from the spleen during electrical stimulation (1, 2). In other studies similar measurements were made using perfused muscle beds (3, 4). These studies gave useful information on the total amounts of norepinephrine released rather than the concentrations of the amine attained in the region of the vascular smooth muscle receptors. However, in two of the studies (3, 4) a calculation was made of concentrations of norepinephrine in the immediate vicinity of the sympathetic nerves. The estimated concentrations were on the order of 0.5-1 μg/ml. Ljung (5) estimated concentrations of norepinephrine in venous smooth muscle during nerve stimulation, and the values reported ranged from 8 ± 4 × 10⁻₆ M to 2 ± 0.5 × 10⁻₆ M over a stimulation frequency range of 4 to 16 pulses/sec.

In the present study the estimated concentrations of norepinephrine in arterial smooth muscle were lower than those reported by Ljung (5), but they probably reflect the high sensitivity of the isolated rabbit ear artery to norepinephrine. Furthermore, a feature of the present study was that the exogenous and the endogenous norepinephrine followed similar pathways in reaching the receptor sites, thus reducing the number of factors which could influence the final concentrations of norepinephrine from each source.

It is recognized that the estimation of norepinephrine concentrations by the methods employed in this study is subject to error, but the factors considered above which could affect amine concentrations probably are not sufficient to cause large losses of amine in its path to the smooth muscle receptors. Thus the estimated concentrations are probably not far removed from those actually responsible for constriction of the smooth muscle of the rabbit ear artery during sympathetic nerve stimulation.

Acknowledgment

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References


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